

DRUG RESISTANT MALARIA: BEYOND ARTEMISININ A CHALLENGE TO MEDICAL SCIENCE

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ABSTRACT

Malaria is caused by four species of *Plasmodium* the fifth *P.knowlesi* is prevalent in Malaysia and Southeast Asia. Malaria due to *Plasmodium falciparum* has developed resistant to all first line antimalarial drugs. Chloroquine has been replaced by Sulfadoxine-pyrimethamine (SP) as the first- line treatment of uncomplicated malaria. Resistance to chloroquine SP combination is already reported in Africa, making this combination unsuitable for use in Africa. Chloroquine and SP are replaced by artemisinin combination therapies (ACTs) which are more effective. The emergence of resistance to artemisinin derivatives has increased recently with reports of treatment failures with artesunate-mefloquine and artemether-lumefantrine in Thai Cambodian malaria control programs. The current generation of ACTs will not maintain the efficacy indefinitely. Malaria control programs and researchers must join efforts to apply in coordinated proactive monitoring programs to detect the emergence and prevent the spread of resistance to ACTs.

KEYWORDS: Malaria, Sulfadoxine, Pyrimethamine, Artesunate Combination Therapies

INTRODUCTION

Malaria parasites *Plasmodium* first observed in a blood sample by Alphonse Laveran in 1880[1]. Other three species were discovered by other scientists, *Plasmodium vivax* (Grassi and Felette, 1890), *Plasmodium falciparum* (Welch, 1897), and *Plasmodium ovale* (Stephens, 1922). A fifth species of malaria *P.knowlesi* was first time reported in humans (Robert Knowles *et al*, 1932). It is estimated that at least 250 to 500 million febrile illnesses and up to a million deaths annually [2]. With the introduction of chloroquine and dichlorodiphenyltrichloroethane (DDT) at the end of World War II brought new power to malaria control efforts.[3]. With the massive use of chloroquine in the 1980s selected for chloroquine-resistant *Plasmodium falciparum* strains that entered and spread in Africa.

The impact of chloroquine resistance was especially evident in young children [4,5]. Chloroquine – resistant *P.falciparum* malaria is wide spread in sub-Saharan Africa, Asia, and Latin America. It has also been reported in areas of the Middle East, including Iran, Yemen, Oman and Saudi Arabia [6,7], but not from Mexico, other regions in Central America west of Panama Canal, Haiti, or the Dominican Republic. High-grade resistance of *P.vivax* malaria to chloroquine has been reported in Oceania and parts of Southeast Asia [8,9]. *P.vivax* malaria not responding to chloroquine treatment have also been reported from Brazil, Guyana, Colombia, Peru, India, and Myanmar[10-15].

Artemisinin-resistant *P.falciparum*, in Asia and Africa. Mefloquine-resistant *P.falciparum* malaria in Thailand, in Thailand, Cambodia, Myanmar and Vietnam[16-18]. The emergence of resistance to artemisinin derivatives have increased recently with reports of treatment failures with artesunate-mefloquine and artemether-lumefantrine in Thai Cambodian malaria control programs [19]. Emergence of resistance to artemisinin-a hallmark benefit of artemisinin in the treatment of severe malaria, may become less dependable after artemisinin dosing in Southeast Asia [20].

This paper reviews the resistance to antimalarial drugs with emphasis on resistance to artemisinin which has become less reliable.

RESISTANCE TO ANTIMALARIAL DRUGS

Mechanism of Action

Chloroquine

Intraerythrocytic parasites consume the hemoglobin of their host cells, breaking down it with in a large digestive food vacuole and releasing hemoglobin molecules (heme) that are poisonous if not detoxified. Malaria parasites normally allow these heme molecules to polymerize into inert crystals called hemozoin that can be visualized by light microscopy as intraerythrocytic pigment in thin blood smears. Chloroquine acts by forming toxic complexes with heme molecules and interfering with their crystallization [21].

This mechanism of action explains why chloroquine is effective against developing intraerythrocytic trophozoites but ineffective against other parasite stages i.e. mature gametocytes, liver schizonts that do not actively consume hemoglobin. Chloroquine-resistant *P. falciparum* reduce the amount of drug that accumulates in their digestive vacuoles [22].

The mechanism involves mutations in a conserved transport molecule of the digestive vacuole membrane termed PfCRT (*P. falciparum* chloroquine resistance transporter) [23]. The mutation include a key change from lysine to threonine in the 76th amino acid (K76T) plus additional mutations that depend on their geographic origin [24,25]. Drug selection for mutant PfCRT is evident in association of the K76T marker with increased plasma chloroquine levels and with treatment failures in children receiving drug [26]. Several lines of evidence now indicate that chloroquine resistance involves a specific interaction between chloroquine and the modified form of PfCRT that promotes drug efflux from digestive vacuole [27,28].

While PfCRT is the central determinant of chloroquine resistance, other host and parasite factors also influence treatment outcomes. For example, clearance of phenotypically chloroquine resistant parasites can occur after chloroquine treatment and become increasingly prevalent in children as they grow older, presumably owing to the immunity that develops from repeated episodes of malaria [29]. Parasite transport modules in addition to PfCRT have also been proposed to modulate or contribute to the ability of chloroquine-resistant parasites to cope with the drug [30].

Sulfadoxone-Pyrimethamine

Dihydropterotate synthase (DHPS) and dihydrofolate reductase (DHFR) are sequentially involved in the folate pathway of nucleic acid synthesis. Pyrimethamine inhibits parasite DHFR and the production of tetrahydrofolate, an essential cofactor for one-carbon metabolism required for the synthesis of nucleic acid and certain amino acids. The substitution of asparagine for serine in position 108 in DHFR is critical for the initial development of pyrimethamine resistance, with additional mutation (Ile51, Arg59, Leu 164) increasing the degree of pyrimethamine resistance [31]. Part of the sulfadoxine's action is thought to be inhibition of parasites DHPS and point mutations in DHPS reduce its affinity for sulfadoxine [31]. Analysis of the mutant *dhfr* and *dhps* alleles in field studies supports conclusions that clinically significant resistance to pyrimethamine arises from multiple mutations in *dhfr* and *dhps* and *dhps* mutations are likely selected after mutations in *dhfr* are already present [32].

Atovaquone-Proguanil-Malarone

Atovaquone binds cytochrome b and inhibits parasites mitochondrial electron transport, leading to collapse of the

mitochondrial membrane potential [33, 34]. This effect is potential by proguanil. The substitution of serine for tyrosine at condon 268 of the cytochrome b gene is associated with resistance to atovaquone and AP combination [35, 23]. Cyotoguanil the active metabolite of proguanil, inhibits DHFR. Point mutations in *dhfr* resistance to cyotoguanil [36].

Doxycycline

Doxycycline inhibits protein synthesis elongation binding of aminocyl-tRNA to ribosome 30S subunit. Resistance to human malaria parasites have not been described. Doxycycline is successfully used as malaria prophylaxis in IrianJaya [37].

Mefloquine, Qunidine and Quinine

These three antimalarial drugs, mefloquine, quinidine, and quinine are thought to form complex toxic to the parasite by binding to heme. Mefloquine resistance may be associated in part with increases in expression and mutations in the P-glycoprotein homolog-1 gene *pfmdr1* [38]. Decreased quinine sensitivity is associated with resistance to other structurally related drugs such as melfoquine and halofantrine, suggesting that drug resistance mechanism may share various genetic determinants [39]. Some studies have implicated *pfmdr1* mutations in mefloquine, quinine, and halofantrine resistance and *pfcr* mutations in quinine and quinidine responses [40]. The different level of quinine susceptibility among parasites and the relatively slow rate at which quinine resistance has spread throughout the world indicate that quinine resistance is a complex phenotype and is probably affected by other genes in addition to *pfmdr1*. The results of a linkage analysis and surveys of parasites from Southeast Asia, Africa, and South America support a model in which multiple genes can combine in different ways to produce similar phenotypes of reduce quinine response [40].

Artemisinin Derivatives

At present high level of resistance to the artemisinin derivatives has not been found with clinical samples, successful selection of rodent malaria parasites strains with reduced susceptibility, and reports of *P.falciparum* strains with prolonged clearance times in vivo [41,20], raise concerns that strains of human malaria parasites with significant clinical resistance may evolve and spread. No molecular mechanism to account for artemisinin resistance has been established. An S769N mutation in an ATPase enzyme (PfATPase 6) was proposed as a possible determinant of artemisinin resistance [42]. One study associated elevated IC50s with its mutation in strains of *P.falciparum* from French Guiana, but resistance has not been associated with this mutation in field isolates elsewhere nor has mutation been found in rodent malaria parasites selected for resistance[43,41,20].

CLINICAL MANIFESTATION AND DORMANCY OF MALARIA

The malaria parasite incubation period after and infective mosquito bite includes the time required for parasites to progress through liver schizogony and produce symptoms by their propagation in the blood stream. For primary attacks, this period is typically about 8 to 25 days but may be much longer depending on immune status of the infected person, the strain as well as the species of *Plasmodium*, the dose of sporozoites, and the possible effects of partially effective chemoprophylaxis. Relapses from latent hyponozoite may develop months or years after mosquito bites. Late-onset or recrudescence of *P.falciparum* malaria may also occur in individuals who have suppressed parasitemia of drug resistant parasites with chemoprophylactic drugs [44]. Febrile patients presenting within 7 days of entering an endemic area are likely to have malaria, unless there has been earlier exposure to infective mosquito bites.

As a general rule, and because of danger of acute *P.falciparum* infection, all travelers who have visited a malaria-endemic area in the 3 months prior to onset of fever or other suggestive symptoms should be considered to have malaria

until proven otherwise. Even in patients beyond this time frame, it is wise to consider *P.falciparum* malaria, for example, in the recent report of a symptomatic presentation in an 18 years-old patient with sickle cell disease 4 years after visiting an endemic area [45]. There is firm experimental foundation showing that malaria (*Plasmodium*) may persist for long periods in vivo in a viable state but not multiplying state. Latent attacks from reactivation of *P.vivax*, *P.ovale* hypnozoites usually occur within 3 years and are rare more than 5 years after exposure. Recrudescence *P.malaria* symptoms in individuals with subclinical parasitemia has been reported decades after initial infection [45].

THERAPY OF MULTIDRUG RESISTANT MALARIA

Malaria due to *P.falciparum* can be fatal if not diagnosed and treated promptly and appropriately. This is especially true of nonimmune travelers returning from visits to malaria-endemic areas. Malaria is a disease of protean manifestation [46]. Artemisinin and its derivatives (artesunate, arthemether, dihydroartemisinin) are now commonly used in Africa and Southeast Asia for the treatment of uncomplicated malaria, that caused by multidrug-resistant *P.falciparum* [47]. Parasites recrudescence weeks after therapy with artemisinin does occur, often the elimination of these drugs and recovery of parasitemia without selection of mutant parasites that are truly drug-resistant.

The addition of partner drug (e.g. chloroquine, sulfadoxine-primethamine, or mefloquine) to 3- day course of artemisinin derivative was shown in a meta-analysis to substantially reduce treatment failure and recrudescence [48,49]. Artemisinin derivatives (artemisinin, arthemether, dihydroartemisinin) are derived from *Artemisia annua*, (qinghao) a plant used in China for millennia as therapy for fevers [50]. Artemisinin derivatives are consistently effective against multidrug-resistant parasites and rapid clearance of parasites and clinical improvement usually within 24 to 36 hours. They are well tolerated and safe in adults, children, and pregnant women [51]. Although neurotoxicity can occur with supraphysiologic doses in animals, it has not been documented in humans [52].

P.falciparum, resistant to most standard antimalarial drugs, poses a major problem for the treatment of malaria. Several countries in sub-Saharan Africa have replaced chloroquine with sulfadoxine-pyrimethamine (SP) as the first- line drug for the treatment of uncomplicated *P.falciparum* malaria [53]. In other areas of the world where SP replaced chloroquine, such as South-East Asia, resistance to SP developed within few years of its introduction. In East Africa resistance to SP is present, resulting in a decrease in the effectiveness of this drug [54].

In a study in Gambia, children with uncomplicated *P.falciparum* malaria treated with 3 day of artesunate plus SP had faster resolution of fever, parasite clearance, and gametocyte carriage compared with SP alone [55]. Researchers in Kenya in a randomized, double-blind, placebo-controlled trial, the efficacy, safety and tolerability of artesunate plus SP compared with SP alone in the treatment of uncomplicated *P.falciparum* malaria confirmed that parasite clearance and gametocyte carriage were reduced significantly in both combination groups compared with SP alone. Three day artesunate were required to reduce significantly the risk of treatment failure by day 28. However, the high background rate of parasitological failure with SP may make this combination unsuitable for widespread use in Kenya [56].

Nosten *et al* (1994) studied 652 adults and children with acute uncomplicated *falciparum* malaria on the Thai-Burmese border and found that a single- dose artesunate (4 mg/kg) plus mefloquine (25 mg of base/kg) gave more rapid symptomatic and parasitological responses than high-dose mefloquine alone but did not improve cure rate [57].

Other researchers in Thailand reported that introduction of artesunate-mefloquine combination in selected areas along Thai-Myanmar borders in 1995 is believed to be one of the multiple factors responsible for stabilizing the multidrug-resistance problems in Thailand [18]. Today the treatment of choice is artemisinin-based combination therapies

(ACTs). Resistance to artemisinin - the core component of the combination- has now been identified in Cambodia, Myanmar, Thailand and Viet Nam [58].

PREVENTION OF DRUG RESISTANT MALARIA

The concept that resistance could be delayed or prevented by combining drugs with different targets was first developed in the treatment of tuberculosis, and has been adopted widely for the treatment of HIV, leprosy, and cancer. Artemisinin combinations have been proposed as an option for the treatment of drug-resistant malaria [59,60]. The landscape of antimalarial therapy is changing. With new multilateral support for artemisinin combination therapies (ACTs), highly efficacious alternatives are becoming available to replace less effective drugs [chloroquine and sulfadoxine-pyrimethamine (SP) that are still used widely despite their impaired efficacy.

Combination therapies present new challenges for monitoring resistance and efficacy, as well as new prospects for deterring drug resistance [61]. Methods for measuring parasite growth *in vitro* in the presence of increasing drug concentrations were developed for culture-adapted malaria parasites in controlled laboratory testing [62]. Despite the limitations, *in vitro* assays are increasingly important in the era of ACTs because of the inability to rely on molecular methods for monitoring resistance and absence to date of clinically significant resistance to the artemisinin. The early stages of parasite resistance to individual drugs used in combination therapy regimens may not be clinically apparent because of the action of the partner drug(s).

Clinical studies to monitor efficacy may thus be relatively insensitive for heralding the impending failure of drug combinations. While candidate molecular markers for resistance to artemisinin are being studied [63]. Investigators the cases of chloroquine and antifolates, successfully identified the key resistance genes, which, even if they were not the sole genetic contributors to resistance, are clearly its primary determinants. Candidate gene approaches based on non-malaria homologs or on suspected mechanisms of drug action have been used to study genetic determinants of resistance to drugs included in ACTs.

In an example of the homolog candidate gene approach *in vitro* and clinical evidence suggests that increased *pfmdr1* copy number is associated with resistance to mefloquine, and artemisinin, as well as other antimalarial drugs [64,65]. ACTs, with their rapid action and excellent efficacy, are being embraced by the policy makers throughout the Africa (and in other malaria endemic areas). However, the current generation of ACTs will not maintain their efficacy indefinitely. Researchers and malaria control workers of all stripes must join efforts to apply *in vitro*, molecular, genomic, pharmacokinetic, and clinical methods in coordinated proactive monitoring programs to detect emergence and deter the spread of resistance to ACTs [61].

CONCLUSIONS

P. falciparum has developed resistance to all the first line used antimalarial drugs. Artesunate SP combinations are also not the drugs of choice anymore. Resistance to artemisinin combinations is also reported in Southeast Asia. Artemisinin combination therapies will not maintain their effectiveness indefinitely. Efforts must be made to detect and prevent the spread of resistance to ACTs.

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